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**The  
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- 1 Please give the title of the invention      PRODUCTION OF RECOMBINANT CHIMERIC PROTEINS FOR VACCINE USE

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Country (and State of incorporation, if appropriate)      Canada

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PRODUCTION OF RECOMBINANT CHIMERIC PROTEINS  
FOR VACCINE USE

5 The present invention relates to the engineering and expression of chimeric genes, particularly those containing sequences from the genes coding for the major immunogenic proteins of both human Parainfluenza virus (PIV) and Respiratory syncytial virus (RSV). The present invention also relates to the formulation of various recombinant PIV/RSV immunogens to produce safe and efficacious vaccines capable of protecting infants and young children against infection with both PIV and RSV.

10 Human Parainfluenza virus types 1, 2, 3 and Respiratory syncytial virus types A and B are the major viral pathogens responsible for causing severe respiratory tract infections in infants and young children. Safe and effective vaccines for protecting infants against these viral infections are not available and are urgently required. It is anticipated that the development of a single recombinant immunogen capable of simultaneously protecting infants against infection with both Parainfluenza and Respiratory syncytial viruses could significantly reduce the morbidity and mortality caused by these viral infections.

25 Identification of the major immunogenic proteins of RSV and PIV has provided the scientific basis for designing the chimeric RSV/PIV immunogens described herein. It has been reported that a protective response is contingent on the induction of neutralizing antibodies against the major viral surface glycoproteins. For PIV, these protective immunogens are the HN protein which possesses both hemagglutination and neuraminidase activities and the fusion (F) protein, which is responsible for both fusion of the virus to the host cell membrane and cell-to-cell spread of the virus. For RSV, the two major immunogenic proteins are the 80-90 kDa G glycoprotein and the 70 kDa fusion (F) protein.

The G and F proteins are thought to be functionally analogous to the PIV HN and F proteins, respectively.

In accordance with the present invention, the inventors have specifically engineered several model  
5 PIV/RSV chimeric genes containing relevant sequences from selected genes coding for the PIV-3 and RSV surface glycoproteins. All genes in the chimeric constructs were obtained from recent clinical isolates of PIV-3 and RSV. The chimeric gene constructs include gene  
10 sequences from either PIV-3 F or HN genes linked to either RSV G (subtypes A and B) or F genes in all possible relative orientations and combinations.

The constructs may consist of either the entire gene sequences or gene segments coding for immunogenic  
15 epitopes thereof. In addition, the present invention also includes trimeric gene constructs containing the PIV and RSV genes or gene segments linked in all possible relative orientations. For example:

20 FPIV - HNPIV - F or GRSV  
FPIV - FRSV - GRSV  
HNPIV - FRSV - GRSV

The chimeric and trimeric genes are sub-cloned into appropriate vectors for expression in both mammalian and insect cells. Alternatively, recombinant poxviruses and  
25 transformed mycobacteria (BCG) can be used for immunization. Chimeric PIV/RSV proteins present in either the supernatants or cell lysates of transfected cells then are purified by a combination of conventional chromatographic procedures. To evaluate the  
30 immunogenicity and protective ability of the combinant proteins, guinea pigs, hamsters and cotton rats are immunized with either recombinant BCG or poxviruses or with varying doses of the purified chimeric PIV/RSV proteins administered in the presence of an appropriate  
35 adjuvant, such as aluminum phosphate. In an attempt to further enhance the immunoprotective ability of the

Functionally active, the model res

chimeric proteins, the recombinant antigen may contain or be supplemented with other immunogenic proteins of PIV and RSV produced either by genetic engineering techniques or purified from the virus by a series of chromatographic procedures. The final vaccine preparation, when formulated with aluminum phosphate as an adjuvant, can be used as a readily injectable preparation for protecting humans against infection with both PIV-3 and RSV. The invention also includes the use of delivery systems, such as iscoms and liposomes, as well as adjuvants other than aluminum phosphate. The effectiveness of the invention is not limited to the preparation of recombinant chimeric PIV-3 and RSV proteins, but is applicable to the production of chimeric immunogens composed of either the entire sequences or regions of the major immunogenic regions from other Paramyxoviruses linked in tandem.

#### EXAMPLES

Methods for cloning and sequencing the PIV-3 and RSV genes as well as the procedures for sub-cloning the genes into appropriate vectors and expressing the gene constructs in mammalian and insect cells are not explicitly described in this disclosure but are well within the scope of those skilled in the art. The drawings which accompany and form part of this specification are referred to in the Examples.

##### Example 1:

This Example outlines the strategy used to clone and sequence the PIV-3 F, HN and RSV F genes. These genes were used in the construction of the FpIV-3-FRSV and FRSV-HNpIV-3 chimeric genes detailed in Examples 2 to 4 and Example 8, respectively.

Two PIV-3 F gene clones were obtained from cDNA derived from viral RNA extracted from a recent clinical isolate of PIV-3. The PIV-3 HN and RSV F genes were cloned from a cDNA library prepared from mRNA isolated

from MRC-5 cells infected with clinical isolates of either PIV-3 or RSV. The PIV-3 F, HN and RSV F gene clones were sequenced by the dideoxynucleotide chain termination procedure. Sequencing of both strands of the genes was performed by a combination of manual and automated sequencing.

The nucleotide and amino acid sequences of the PIV-3 F gene is presented in Figure 1 and the restriction map of the gene is outlined in Figure 2. Sequence analysis of the 1844 nucleotides of two PCR amplified PIV-3 F gene clones confirmed that the clones were identical. Comparison of the coding sequence of the PCR-amplified PIV-3 F gene clone with that of the published PIV-3 F gene sequence revealed a 2.6% divergence in the coding sequence between the two genes resulting in 14 amino acid substitutions.

Figure 3 shows the nucleotide and amino acid sequences of the PIV-3 HN gene and the restriction map of the gene is presented in Figure 4. Analysis of the 1833 nucleotide sequence from two non-PCR amplified HN clones confirmed that the sequences were identical. A 4.4% divergence in the coding sequence of the PIV-3 HN gene was noted when the sequence was compared to the published PIV-3 HN coding sequence. This divergence resulted in 17 amino acid substitutions in the coding sequence of the non-PCR amplified PIV-3 HN gene.

The nucleotide and amino acid sequences of the RSV F gene is reported in Figure 5 and the restriction map of the gene is shown in Figure 6. Analysis of the 1859 nucleotide sequence from two RSV F clones verified complete sequence homology between the two clones. Comparison of this nucleotide sequence with that reported for the RSV F gene revealed approximately 1.8% divergence in the coding sequence resulting in 11 amino acid substitutions.

The full-length PIV-3 F, HN and RSV F genes were

cloned into the multiple cloning site of a Bluescript-based vector either by blunt end ligation or using appropriate linkers. The cloning vectors containing the PIV-3 F, HN and RSV F genes were named pPIVF, pPIVHN and pRSVF, respectively.

Example 2:

This Example illustrates the construction of a Bluescript-based expression vector containing the chimeric FPIV-3 -FRSV. This chimeric gene construct contained the 5'-untranslated region of the PIV-3 F gene but lacked the hydrophobic anchor and cytoplasmic domains of both the PIV-3 and RSV F genes.

To prepare the PIV-3 portion of the chimeric gene, the full-length PIV-3 gene lacking the transmembrane coding region and cytoplasmic tail was retrieved from plasmid pPIVF by cutting the polylinker with EcoRV and the gene with BsrI. A BsrI-BamHI oligonucleotide cassette (Fig. 7A) containing a PpuMI site and three successive translational stop codons was ligated to the truncated 1.6 Kb EcoRV-BsrI PIV-3 F gene fragment and cloned into the EcoRV-BamHI sites of a bluescript based-expression vector containing the human methallothionein promoter and the poly A and IVS sequences of the SV40 genome to generate plasmid pME1.

To engineer the RSV F gene component of the chimeric construct, the RSV F gene lacking the transmembrane coding region and cytoplasmic tail was retrieved from plasmid pRSVF by cutting the polylinker with EcoRI and the gene with BspHI. A synthetic BspHI-BamHI oligonucleotide cassette (Fig. 7B) containing three successive translational stop codons was ligated to the 1.6 Kb truncated RSV F gene and cloned into the EcoRI-BamHI sites of the Bluescript-based expression vector to produce plasmid ES13A. Plasmid ES13A was then cut with EcoRI and PpuMI to remove the leader and F2 coding sequences from the truncated RSV F gene. The

leader sequence was reconstructed using an EcoRI-PpuI oligocassette (Fig. 7C) and ligated to the RSV F1 gene segment to generate plasmid ES23A.

To prepare the chimeric FPIV-3-FRSV gene, containing the 5'-untranslated region of the PIV-3 F gene linked to the truncated RSV F1 gene fragment, plasmid pME1 (containing the 1.6 Kb truncated PIV-3 F gene) was first cut with PpuMI and BamHI. The 6.2 Kb PpuMI-BamHI restricted pME1 vector was dephosphorylated with intestinal alkaline phosphatase. The 1.1 Kb RSV F1 gene fragment was retrieved from plasmid ES23A by cutting the plasmid with PpuMI and BamHI. The 1.1 Kb PpuMI-BamHI RSV F1 gene fragment was cloned into the PpuMI-BamHI sites of the dephosphorylated pME1 vector to generate plasmid ES29A. This chimeric gene construct contained the 5'-untranslated region of the PIV-3 f gene but lacked the hydrophobic anchor domains and cytoplasmic tails of both the PIV-3 and RSV F genes.

Example 3:

This Example illustrates the construction of a Bluescript-based expression vector containing the PIV-3 F gene lacking both the 5'-untranslated and transmembrane anchor regions.

Plasmid pPIVF containing the full length PIV-3 F gene was cut with BamHI, blunt ended with Klenow polymerase and then cut with BsrI to remove the transmembrane coding region and cytoplasmic tail. The Bluescript-based expression vector (containing the human methallothionein promoter and poly A and IVS sequences of the SV40 genome) was cut with SmaI and BamHI. A synthetic BsrI-BamHI oligonucleotide cassette (Fig. 7D) containing a translational stop codon was ligated with the 1.6 Kb blunt ended-BsrI PIV-3 F gene fragment to the 4.5 Kb SmaI (blunt ended)-BamHI restricted expression vector to produce plasmid pMpFB. The PIV-3 F gene of this construct lacked the transmembrane coding region



but contained the 5'-untranslated region. To engineer a plasmid containing the PIV-3 F gene devoid of both the 5'-untranslated region and the hydrophobic anchor domain, plasmid pMpFB was cut with EcoRI and BstBI. An  
5 EcoRI-BstBI oligocassette (Fig. 7E) containing the sequences to reconstruct the signal peptide and coding sequences removed by the EcoRI-BstBI cut was ligated to the 6.4 Kb EcoRI-BstBI restricted pMpFB vector to produce plasmid pMpFA. The PIV-3 F gene of this  
10 construct lacked both the 5'-untranslated region and the 3'-transmembrane anchor domain.

Example 4:

This Example illustrates the construction of the chimeric F<sub>PIV-3</sub>-F<sub>RSV</sub> gene composed of the truncated PIV-  
15 3 F gene devoid of the 5'-untranslated region linked to the truncated RSV F1 gene.

To prepare this chimeric gene construct, plasmid ES29A (Example 2) was cut with BstBI and BamHI to release the 2.4 Kb BstBI-BamHI PIV-3 F2 + 1-RSV F1  
20 chimeric gene fragment. This BstBI-BamHI chimeric gene fragment was isolated from a low melting point agarose gel and cloned into the BstBI-BamHI sites of the dephosphorylated vector pMpFA to produce plasmid ES60A. This construct contained the PIV-3 F gene lacking both  
25 the 5'-untranslated region and the hydrophobic anchor sequence linked to the F1 coding region of the truncated RSV F gene. This chimeric gene was subsequently subcloned into the baculovirus expression vector (detailed in Example 5).

30 Example 5:

This Example illustrates the construction of the modified pAc 610 baculovirus expression vector containing the native polyhedrin promoter and the chimeric F<sub>PIV-3</sub>-F<sub>RSV</sub> gene consisting of the PIV-3 F gene  
35 lacking both the 5'-untranslated and transmembrane coding sequences linked to the truncated RSV F1 gene.

The pAc 610 baculovirus expression vector was modified to contain the native polyhedrin promoter in the following manner. Vector pAc 610 was cut with EcoRV and BamHI. The 9.4 Kb baculovirus expression vector lacking the EcoRV-BamHI DNA sequence was isolated from a low melting point agarose gel and treated with intestinal alkaline phosphatase. In a 3-way ligation, an EcoRV-EcoRI oligonucleotide cassette (Fig. 8) containing the nucleotides required to restore the native polyhedrin promoter was ligated with the 1.6 Kb EcoRI-BamHI truncated RSV F gene fragment isolated from construct ES13A and the EcoRV-BamHI restricted pAc 610 phosphatased vector to generate plasmid ES47A. To prepare the pAc 610 based expression vector containing the chimeric FpIV-3-FRSV gene, plasmid ES47A was first cut with EcoRI and BamHI to remove the 1.6 Kb truncated RSV F gene insert. The 2.5 Kb FpIV-3-FRSV chimeric gene was retrieved by cutting plasmid ES60A with EcoRI and BamHI. The 2.5 Kb EcoRI-BamHI chimeric gene was ligated to the 7.7 Kb ES47A vector restricted with EcoRI-BamHI to generate plasmid pAc DR7-8.

Example 6:

This Example outlines the preparation of plaque purified recombinant baculoviruses containing the chimeric FpIV-3-FRSV gene.

Spodoptera frugiperda (Sf9) cells were co-transfected with 10  $\mu$ g wild-type AcMNPV DNA and 2.5  $\mu$ g of FpIV-3-FRSV plasmid DNA (construct DR7-8). Putative recombinant baculoviruses (purified once by serial dilution) containing the FpIV-3-FRSV chimeric gene were identified by dot-blot hybridization. Lysates of insect cells infected with the putative recombinant baculoviruses were probed with the  $^{32}$ P-labelled FpIV-3-FRSV chimeric gene insert. Recombinant baculoviruses were plaque-purified twice before being used for expression studies. All procedures were carried out

according to the protocols outlined by Summers and Smith in "A Manual of Methods for Baculovirus Vectors and Insect Cell Culture Procedures".

Example 7:

5        This Example illustrates the presence of the chimeric FpIV-3 - FRSV protein in supernatants and cell lysates of infected Sf9 cells.

10        Insect cells were infected with the plaque purified recombinant baculoviruses at a MOI of 8. Concentrated supernatants from cells infected with the recombinant viruses were positive in a PIV-3 F specific ELISA. In addition, when lysates from <sup>35</sup>S methionine labelled infected cells were subjected to SDS-polyacrylamide gel electrophoresis and gels were analyzed by  
15        autoradiography, a strong band with expected MW of 90 kDa was present in lysates of cells infected with the recombinant viruses but was absent in the lysates from wild type infected cells. The presence of the chimeric FpIV-3 -FRSV protein in the lysates of cells infected  
20        with the recombinant baculoviruses was further confirmed by Western blot analysis using anti-PIV-3 F and anti-RSV F monospecific antisera and/or monoclonal antibodies. Lysates from cells infected with the recombinant baculoviruses reacted with both anti-PIV-3 and anti-RSV  
25        antisera in immunoblots. As shown in the immunoblot of Fig. 9, lysates from cells infected with either the RSV F or FpIV-3-FRSV recombinant baculoviruses reacted positively with the anti-F RSV Mab. As expected, lysates from cells infected with wild type virus did not  
30        react with this Mab. In addition, only lysates from cells infected with the chimeric FpIV-3-FRSV recombinant viruses reacted with the anti-PIV-3 F<sub>1</sub> antiserum.

Example 8:

35        This Example illustrates the construction of a baculovirus expression vector containing the chimeric FRSV-HNpIV-3 gene consisting of the truncated RSV F and

PIV-3 HN genes linked in tandem. In this baculovirus expression vector, designated pD2, the polyhedrin ATG start codon was converted to ATT and the sequence CCG was present downstream of the polyhedrin gene at positions +4, 5, 6. Insertion of a structural gene several base pairs downstream from the ATT codon is known to enhance translation.

To engineer the FRSV-HNpIV-3 gene, the RSV F gene lacking the transmembrane coding region was retrieved from plasmid pRSVF by cutting the polylinker with EcoRI and the gene with BspHI. The PIV-3 HN gene devoid of the hydrophobic anchor domain was retrieved from plasmid pPIVHN by cutting the gene with BspHI and the polylinker with BamHI. The 1.6 Kb EcoRI-BspHI RSV F gene fragment and the 1.7 Kb BspHI-BamHI PIV-3 HN gene fragments were isolated from low melting point agarose gels. For cloning purposes, the two BspHI sites in the Bluescript-based mammalian cell expression vector containing the human methallothionein promoter and poly A and IVS sequences from the SV40 genome were mutated. Mutations were introduced in the BspHI sites of the vector by cutting the expression vector with BspHI, treating both the 1.1 Kb BspHI restricted vector and the 1.1 Kb fragment released by the BspHI cut with Klenow polymerase and ligating the blunt-ended 1.1 Kb fragment to the blunt-ended Bluescript-based expression vector to generate plasmid pM. Since insertion of the 1.1 Kb blunt-end fragment in the mammalian cell expression vector in the improper orientation would alter the  $amp^r$  gene of the Bluescript-based expression vector, only colonies of HB101 cells transformed with the pM plasmid DNA with the 1.1 Kb blunt-ended fragment in the proper orientation could survive in the presence of ampicillin. Plasmid DNA was purified from ampicillin-resistant colonies of HB101 cells transformed with plasmid pM by equilibrium centrifugation in cesium chloride-ethidium

bromide gradients. The 1.6 Kb EcoRI-BspHI RSV F and 1.7 Kb BspHI-BamHI PIV-3 HN gene fragments were ligated via the BspHI site and cloned into the EcoRI-BamHI sites of vector pM to generate plasmid pM RF-HN. To restore specific coding sequences of the RSV F and PIV-3 HN genes removed by the BspHI cut, a BspHI-BspHI oligonucleotide cassette (Fig. 10) containing the pertinent RSV F and PIV-3 HN gene coding sequences was ligated via the BspHI site to the BspHI-restricted plasmid pM RF-HN to produce plasmid pM' RF-HN. Clones containing the BspHI-BspHI oligonucleotide cassette in the proper orientation were identified by sequence analysis of the oligonucleotide linker and its flanking regions. To clone the chimeric FRSV-HNpIV-3 gene into the baculovirus expression vector (pD2) in which the ATG of the polyhedrin start codon was converted to ATT, the FRSV-HNpIV-3 truncated gene was first retrieved from plasmid pM' RF-HN by cutting the plasmid with EcoRI. The 3.3 Kb FRSV-HNpIV-3 gene was then cloned into the EcoRI site of the baculovirus expression vector plasmid pD2 to generate plasmid pD2 RF-HN. Proper orientation of the 3.3 Kb EcoRI FRSV-HNpIV-3 chimeric gene insert in plasmid pD2 RF-HN was confirmed by sequence analysis.

Example 9:

This Example outlines the preparation of plaque-purified recombinant baculoviruses containing the chimeric FRSV-HNpIV-3 gene.

Spodoptera frugiperda (Sf9) cells were co-transfected with 1  $\mu$ g wild-type AcNPV DNA and 2  $\mu$ g of FRSV-HNpIV-3 plasmid DNA (construct pD1RF-HN). Putative recombinant baculoviruses (purified once by serial dilution) containing the FRSV-HNpIV-3 chimeric gene were identified by dot-blot hybridization. Lysates of insect cells infected with the putative recombinant baculoviruses were probed with the  $^{32}$ P-labelled RSV F or PIV-3 HN gene oligonucleotide probes. Recombinant

baculoviruses were plaque-purified three times before being used for expression studies. All procedures were carried out according to the protocols outlined by Summers and Smith in "A Manual of Methods for  
5 Baculovirus Vectors and Insect Cell Culture Procedures".

Example 10:

This Example illustrates the presence of the chimeric FRSV-HNpIV-3 protein in supernatants of infected Sf9 cells.

10 Insect cells, maintained in serum free medium EX401, were infected with the plaque purified recombinant baculoviruses at a MOI of 5 to 10 PFU/cell. Supernatants from cells infected with the recombinant  
15 baculoviruses tested positive for expressed protein in both the RSV-F and PIV-3 HN specific ELISAs. In addition, supernatants from infected cells reacted positively with an anti-F RSV monoclonal antibody in immunoblots. A distinct band of approximately 100 kDa  
20 was present in the immunoblots. These results confirm the secretion of the chimeric FRSV-HNpIV-3 protein into the supernatant of Sf9 cells infected with the recombinant baculoviruses.

It will be apparent from the foregoing disclosure, as illustrated by the Examples, that the inventors have  
25 disclosed, in this application, the novel idea of determining the genes in two or more viruses, that are responsible for given antigenic and protective proteins, and joining these together such that, when expressed in a cell system, the resulting product is a chimeric  
30 protein that contains the antigenic proteins and which can be used as a vaccine to protect against disease.

The invention has specified proteins and genes from parainfluenza virus and respiratory syncytial virus that are protective when used as immunisation agents, but the  
35 invention is not limited to these proteins and the organisms that they have come from. The invention may

be applied to any protein that can be shown to be protective and that can be isolated from any organism, whether bacterial or viral. Modifications are possible within the scope of this invention.

FIGURE 1

A G T C A A T A C C A A C A A C T A T T A G C A G T C A T  
T C A G T T A T G G T T G T T G A T A A T C G T C A G T A  
10 20 30

A C G T G C A A G A A C A A G A A A G A A G A G A T T C A A  
T G C A C G T T C T T G T T C T T T C T T C T C T A A G T T  
40 50 60

A A A G C T A A A T A A G A G A A A T C A A A A C A A A A G  
T T T C G A T T T A T T C T C T T T A G T T T T G T T T T C  
70 80 90

G T A T A G A A C A C C C G A A C A A C A A A A T C A A A A  
C A T A T C T T G T G G G C T T G T T G T T T T A G T T T T  
100 110 120

C A T C C A A T C C A T T T T A A A C A A A A A T T C C A A  
G T A G G T T A G G T A A A A T T T G T T T T T A A G G T T  
130 140 150

A A G A G A C C G G C A A C A C A A C A A G C A C C A A A C  
T T C T C T G G C C G T T G T G T T G T T C G T G G T T T G  
160 170 180

← MET PRO THR **LEU** ILE LEU LEU ILE ILE  
A C A A T G C C A A C T T T A A T A C T G C T A A T T A T T  
T G T T A C G G T T G A A A T T A T G A C G A T T A A T A A  
190 200 210

SP → THR THR MET ILE MET ALA **SER** SER CYS GLN  
A C A A C A A T G A T T A T G G C A T C T T C C T G C C A A  
T G T T G T T A C T A A T A C C G T A G A A G G A C G G T T  
220 230 240

ILE ASP ILE THR LYS LEU GLN HIS VAL GLY  
A T A G A T A T C A C A A A A C T A C A G C A T G T A G G T  
T A T C T A T A G T G T T T T G A T G T C G T A C A T C C A  
250 260 270

VAL LEU VAL ASN SER PRO LYS GLY MET LYS  
G T A T T G G T C A A C A G T C C C A A A G G G A T G A A G  
C A T A A C C A G T T G T C A G G G T T T C C C T A C T T C  
280 290 300



SER GLN ASN PHE GLU THR ARG TYR LEU  
A T C A C A A A A C T T C G A A A C A A G A T A T C T A  
A T A G T G T T T T G A A G C T T T G T T C T A T A G A T  
310 320 330

ILE LEU SER LEU ILE PRO LYS ILE GLU ASP  
A T T T T G A G C C T C A T A C C A A A A A T A G A A G A C  
T A A A A C T C G G A G T A T G G T T T T T A T C T T C T G  
340 350 360

SER ASN SER CYS GLY ASP GLN GLN ILE LYS  
T C T A A C T C T T G T G G T G A C C A A C A G A T C A A A  
A G A T T G A G A A C A C C A C T G G T T G T C T A G T T T  
370 380 390

GLN TYR LYS ARG LEU LEU ASP ARG LEU ILE  
C A A T A C A A G A G G T T A T T G G A T A G A C T G A T C  
G T T A T G T T C T C C A A T A A C C T A T C T G A C T A G  
400 410 420

ILE PRO LEU TYR ASP GLY LEU ARG LEU GLN  
A T C C C T C T A T A T G A T G G A T T A A G A T T A C A G  
T A G G G A G A T A T A C T A C C T A A T T C T A A T G T C  
430 440 450

LYS ASP VAL ILE VAL THR ASN GLN GLU SER  
A A A G A T G T G A T A G T A A C C A A T C A A G A A T C C  
T T T C T A C A C T A T C A T T G G T T A G T T C T T A G G  
460 470 480

ASN GLU ASN THR ASP PRO ARG THR ARG  
A A T G A A A A C A C T G A T C C C A G A A C A A G A C G A  
T T A C T T T T G T G A C T A G G G T C T T G T T C T G C T  
490 500 510

F2-F1 cleavage site  
SER PHE GLY GLY VAL ILE GLY THR ILE ALA  
T C C T T T G G A G G G G T A A T T G G A A C C A T T G C T  
A G G A A A C C T C C C C A T T A A C C T T G G T A A C G A  
520 530 540

LEU GLY VAL ALA THR SER ALA GLN ILE THR  
C T G G G A G T A G C A A C C T C A G C A C A A A T T A C A  
G A C C C T C A T C G T T G G A G T C G T G T T T A A T G T  
550 560 570

ALA ALA VAL ALA LEU VAL GLU ALA LYS GLN  
G C G G C A G T T G C T C T G G T T G A A G C C A A G C A G  
C G C C G T C A A C G A G A C C A A C T T C G G T T C G T C  
580 590 600

ALA LYS SER ASP ILE GLU LYS LEU LYS GLU  
G C A A A A T C A G A C A T C G A A A A A C T C A A A G A A  
C G T T T T A G T C T G T A G C T T T T T G A G T T T C T T  
610 620 630

ALA ILE ARG ASP THR ASN LYS ALA VAL GLN  
G C A A T C A G G G A C A C A A A C A A A G C A G T G C A G  
C G T T A G T C C C T G T G T T T G T T T C G T C A C G T C  
640 650 660

SER VAL GLN SER SER ILE GLY ASN LEU ILE  
T C A G T T C A G A G C T C T A T A G G A A A T T T A A T A  
A G T C A A G T C T C G A G A T A T C C T T T A A A T T A T  
670 680 690

VAL ALA ILE LYS SER VAL GLN ASP TYR VAL  
G T A G C A A T T A A A T C A G T C C A A G A T T A T G T C  
C A T C G T T A A T T T A G T C A G G T T C T A A T A C A G  
700 710 720

ASN ASN GLU MET VAL PRO SER ILE ALA ARG  
A A C A A C G A A A T G G T G C C A T C G A T T G C T A G A  
T T G T T G C T T T A C C A C G G T A G C T A A C G A T C T  
730 740 750

LEU GLY CYS GLU ALA ALA GLY LEU GLN LEU  
C T A G G T T G T G A A G C A G C A G G A C T T C A A T T A  
G A T C C A A C A C T T C G T C G T C C T G A A G T T A A T  
760 770 780

GLY ILE ALA LEU THR GLN HIS TYR SER GLU  
G G A A T T G C A T T A A C A C A G C A T T A C T C A G A A  
C C T T A A C G T A A T T G T G T C G T A A T G A G T C T T  
790 800 810

LEU THR ASN ILE PHE GLY ASP ASN ILE GLY  
T T A A C A A A C A T A T T T G G T G A T A A C A T A G G A  
A A T T G T T T G T A T A A A C C A C T A T T G T A T C C T  
820 830 840

SER LEU GLN GLU LYS GLY ILE LYS LEU GLN  
T C G T T A C A A G A A A A A G G A A T A A A A T T A C A A  
A G C A A T G T T C T T T T T C C T T A T T T T A A T G T T  
850 860 870

GLY ILE ALA SER LEU TYR ARG THR ASN ILE  
G G T A T A G C A T C A T T A T A C C G C A C A A A T A T C  
C C A T A T C G T A G T A A T A T G G C G T G T T T A T A G  
880 890 900

THR GLU ILE PHE THR THR SER THR VAL ASP  
 A C A G A A A T A T T C A C A A C A T C A A C A G T T G A T  
 T G T C T T T A T A A G T G T T G T A G T T G T C A A C T A  
 910 920 930

LYS TYR ASP ILE TYR ASP LEU LEU PHE THR  
 A A A T A T G A T A T C T A T G A T C T A T T A T T T A C A  
 T T T A T A C T A T A G A T A C T A G A T A A T A A A T G T  
 940 950 960

GLU SER ILE LYS VAL ARG VAL ILE ASP VAL  
 G A A T C A A T A A A G G T G A G A G T T A T A G A T G T T  
 C T T A G T T A T T T C C A C T C T C A A T A T C T A C A A  
 970 980 990

ASP LEU ASN ASP TYR SER ILE THR LEU GLN  
 G A T T T G A A T G A T T A C T C A A T C A C C C T C C A A  
 C T A A A C T T A C T A A T G A G T T A G T G G G A G G T T  
 1000 1010 1020

VAL ARG LEU PRO LEU LEU THR ARG LEU LEU  
 G T C A G A C T C C C T T T A T T A A C T A G G C T G C T G  
 C A G T C T G A G G G A A A T A A T T G A T C C G A C G A C  
 1030 1040 1050

ASN THR GLN ILE TYR LYS VAL ASP SER ILE  
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 T T G T G A G T C T A G A T G T T T C A T C T A A G G T A T  
 1060 1070 1080

SER TYR ASN ILE GLN ASN ARG GLU TRP TYR  
 T C A T A T A A T A T C C A A A A C A G A G A A T G G T A T  
 A G T A T A T T A T A G G T T T T G T C T C T T A C C A T A  
 1090 1100 1110

ILE PRO LEU PRO SER HIS ILE MET THR LYS  
 A T C C C T C T T C C C A G C C A T A T C A T G A C G A A A  
 T A G G G A G A A G G G T C G G T A T A G T A C T G C T T T  
 1120 1130 1140

GLY ALA PHE LEU GLY GLY ALA ASP VAL LYS  
 G G G G C A T T T C T A G G T G G A G C A G A T G T C A A G  
 C C C C G T A A A G A T C C A C C T C G T C T A C A G T T C  
 1150 1160 1170

GLU CYS ILE GLU ALA PHE SER SER TYR ILE  
 G A A T G T A T A G A A G C A T T C A G C A G T T A T A T A  
 C T T A C A T A T C T T C G T A A G T C G T C A A T A T A T  
 1180 1190 1200

PRO SER ASP PRO GLY PHE VAL LEU ASN  
G C C C T T C T G A T C C A G G A T T T G T A C T A A A C  
C G G G A A G A C T A G G T C C T A A A C A T G A T T T G  
1210 1220 1230

HIS GLU MET GLU SER CYS LEU SER GLY ASN  
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G T A C T T T A C C T C T C G A C G A A T A G T C C T T T G  
1240 1250 1260

ILE SER GLN CYS PRO ARG THR THR VAL THR  
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T A T A G G G T T A C A G G T T C T T G G T G C C A G T G T  
1270 1280 1290

SER ASP ILE VAL PRO ARG TYR ALA PHE VAL  
T C A G A C A T T G T T C C A A G A T A T G C A T T T G T C  
A G T C T G T A A C A A G G T T C T A T A C G T A A A C A G  
1300 1310 1320

ASN GLY GLY VAL VAL ALA ASN CYS ILE THR  
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T T A C C T C C T C A C C A A C G T T T G A C A T A T T G T  
1330 1340 1350

THR THR CYS THR CYS ASN GLY ILE ASP ASN  
A C C A C C T G T A C A T G C A A C G G A A T C G A C A A T  
T G G T G G A C A T G T A C G T T G C C T T A G C T G T T A  
1360 1370 1380

ARG ILE ASN GLN PRO PRO ASP GLN GLY VAL  
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T C T T A G T T A G T T G G T G G A C T A G T T C C T C A T  
1390 1400 1410

LYS ILE ILE THR HIS LYS GLU CYS ASN THR  
A A A A T T A T A A C A C A T A A A G A A T G T A A T A C A  
T T T T A A T A T T G T G T A T T T C T T A C A T T A T G T  
1420 1430 1440

ILE GLY ILE ASN GLY MET LEU PHE ASN THR  
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T A T C C A T A G T T G C C T T A C G A C A A G T T A T G T  
1450 1460 1470

ASN LYS GLU GLY THR LEU ALA PHE TYR THR  
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T T A T T T C T T C C T T G A G A A C G T A A G A T G T G T  
1480 1490 1500

PRO ASN ASP ILE THR LEU ASN ASN SER VAL  
C C A A A T G A T A T A C A C T A A A T A A T T C T G T T  
G G T T T A C T A T A T T G T G A T T T A T T A A G A C A A  
1510 1520 1530

ALA LEU ASP PRO ILE ASP ILE SER ILE GLU  
G C A C T T G A T C C A A T T G A C A T A T C A A T C G A G  
C G T G A A C T A G G T T A A C T G T A T A G T T A G C T C  
1540 1550 1560

LEU ASN LYS ALA LYS SER ASP LEU GLU GLU  
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G A A T T G T T T C G G T T T A G T C T A G A T C T T C T T  
1570 1580 1590

SER LYS GLU TRP ILE ARG ARG SER ASN GLN  
T C A A A A G A A T G G A T A A G A A G G T C A A A T C A A  
A G T T T T C T T A C C T A T T C T T C C A G T T T A G T T  
1600 1610 1620

LYS LEU ASP SER ILE GLY ASN TRP HIS GLN  
A A A C T A G A T T C T A T T G G A A A C T G G C A T C A A  
T T T G A T C T A A G A T A A C C T T T G A C C G T A G T T  
1630 1640 1650

SER SER THR THR ILE ILE ILE ILE LEU ILE  
T C T A G C A C T A C A A T C A T A A T T A T T T T A A T A  
A G A T C G T G A T G T T A G T A T T A A T A A A A T T A T  
1660 1670 1680

MET ILE ILE ILE LEU PHE ILE ILE ASN VAL  
A T G A T C A T T A T A T T G T T T A T A A T T A A T G T A  
T A C T A G T A A T A T A A C A A A T T A A T T A C A T  
1690 1700 1710

THR ILE ILE THR ILE ALA ILE LYS TYR TYR  
A C G A T A A T T A C A A T T G C A A T T A A G T A T T A C  
T G C T A T T A A T G T T A A C G T T A A T T C A T A A T G  
1720 1730 1740

ARG ILE GLN LYS ARG ASN ARG VAL ASP GLN  
A G A A T T C A A A A G A G A A A T C G A G T G G A T C A A  
T C T T A A G T T T T C T C T T T A G C T C A C C T A G T T  
1750 1760 1770

ASN ASP LYS PRO TYR VAL LEU THR ASN LYS  
A A T G A C A A G C C A T A T G T A C T A A C A A A C A A A  
T T A C T G T T C G G T A T A C A T G A T T G T T T G T T T  
1780 1790 1800

\*\*\*\*  
 T G A C A T A T C T A T A G A T C A T T A G A T A T T A A A  
 A C T G T A T A G A T A T C T A G T A A T C T A T A A T T T  
 1810 1820 1830

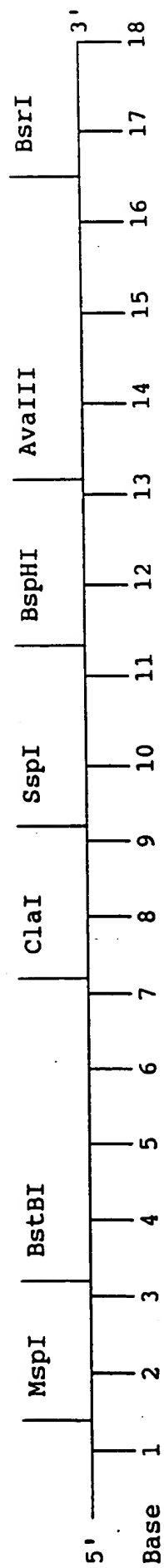
HN gene -----

A T T A T A A A A A A C T T A G G A G T A A A G T T A C G C  
 T A A T A T T T T T T T G A A T C C T C A T T T C A A T G C G  
 1840 1850 1860

A A T C C A A C T C T A C T C A T A T A A T T G A C G G G  
 T T A G G T T G A G A T G A G T A T A T T A A C T G C C C  
 1870 1880

Figure 1: Nucleotide and amino acid sequences of the PIV-3 F gene. The cDNA sequence is shown in the plus (mRNA) strand sense in the 5' to 3' direction. The signal peptide (SP) and the transmembrane (TM) anchor domain are underlined. The predicted F2-F1 cleavage site is indicated by the arrow ( ↓ ). Amino acids differing from the published coding sequence of the PIV-3 F gene are boxed.

FIGURE 2: RESTRICTION MAP OF THE PIV-3 F GENE



1.0 cm = 100 bases

FIGURE 3.

										MET	GLU	TYR	TRP																			
A	G	A	C	A	A	T	C	C	A	A	A	T	T	C	G	A	G	A	T	G	G	A	A	T	A	C	T	G				
T	C	T	G	T	T	T	A	G	G	T	T	T	A	A	G	C	T	C	T	A	C	C	T	T	A	T	G	A	C			
										10											20											30
										LYS	HIS	THR	ASN	HIS	GLY	LYS	ASP	ALA	GLY													
G	A	A	G	C	A	T	A	C	C	A	A	T	C	A	C	G	G	A	A	A	G	A	T	G	C	T	G	G				
C	T	T	C	G	T	A	T	G	G	T	T	A	G	T	G	C	C	T	T	C	C	T	A	C	G	A	C	C				
										40											50											60
										ASN	GLU	LEU	GLU	THR	SER	MET	ALA	THR	ASN													
C	A	A	T	G	A	G	C	T	G	G	A	G	A	C	G	T	C	C	A	T	G	G	C	T	A	C	T	A	A			
G	T	T	A	C	T	C	G	A	C	C	T	C	T	G	C	A	G	G	T	A	C	C	G	A	T	G	A	T	T			
										70											80											90
										GLY	ASN	LYS	LEU	THR	ASN	LYS	ILE	THR	TYR													
T	G	G	C	A	A	C	A	A	G	C	T	C	A	C	C	A	A	T	A	A	G	A	T	A	A	C	A	T	A			
A	C	C	G	T	T	G	T	T	C	G	A	G	T	G	G	T	T	A	T	T	C	T	A	T	T	G	T	A	T			
										100											110											120
										TM																						
										ILE	LEU	TRP	THR	ILE	ILE	LEU	VAL	LEU	LEU													
T	A	T	A	T	T	A	T	G	G	A	C	A	A	T	A	A	T	C	C	T	G	G	T	G	T	T	A	T	T			
A	T	A	T	A	A	T	A	C	C	T	G	T	T	A	T	T	A	G	G	A	C	C	A	C	A	A	T	A	A			
										130											140											150
										SER	ILE	VAL	PHE	ILE	ILE	VAL	LEU	ILE	ASN													
A	T	C	A	A	T	A	G	T	C	T	T	C	A	T	C	A	T	A	G	T	G	C	T	A	A	T	T	A	A			
T	A	G	T	T	A	T	C	A	G	A	A	G	T	A	G	T	A	T	C	A	C	G	A	T	T	A	A	T	T			
										160											170											180
										SER	ILE	LYS	SER	GLU	LYS	ALA	HIS	GLU	SER													
T	T	C	C	A	T	C	A	A	A	A	G	T	G	A	A	A	A	G	G	C	T	C	A	T	G	A	A	T	C			
A	A	G	G	T	A	G	T	T	T	T	C	A	C	T	T	T	T	C	C	G	A	G	T	A	C	T	T	A	G			
										190											200											210
										LEU	LEU	GLN	ASP	ILE	ASN	ASN	GLU	PHE	MET													
A	T	T	G	C	T	G	C	A	A	G	A	C	A	T	A	A	A	T	A	A	T	G	A	G	T	T	T	A	T			
T	A	A	C	G	A	C	G	T	T	C	T	G	T	A	T	T	T	A	T	T	A	C	T	C	A	A	A	T	A			
										220											230											240
										GLU	ILE	THR	GLU	LYS	ILE	GLN	MET	ALA	SER													
G	G	A	A	A	T	T	A	C	A	G	A	A	A	A	G	A	T	C	C	A	A	A	T	G	G	C	A	T	C			
C	C	T	T	T	A	A	T	G	T	C	T	T	T	T	C	T	A	G	G	T	T	T	A	C	C	G	T	A	G			
										250											260											270
										ASP	ASN	THR	ASN	ASP	LEU	ILE	GLN	SER	GLY													
G	G	A	T	A	A	T	A	C	C	A	A	T	G	A	T	C	T	A	A	T	A	C	A	G	T	C	A	G	G			
C	C	T	A	T	T	A	T	G	G	T	T	A	C	T	A	G	A	T	T	A	T	G	T	C	A	G	T	C	C			
										280											290											300



VAL ASN THR ARG LEU LEU THR ILE GLN SER  
A G T G A A T A C A A G G C T T C T T A C A A T T C A G A G  
T C A C T T A T G T T C C G A A G A A T G T T A A G T C T C 330  
310 320

HIS VAL GLN ASN TYR ILE PRO ILE SER LEU  
T C A T G T C C A G A A T T A T A T A C C A A T A T C A C T  
A G T A C A G G T C T T A A T A T A T G G T T A T A G T G A 360  
340 350

THR GLN GLN MET SER ASP LEU ARG LYS PHE  
G A C A C A A C A G A T G T C A G A T C T T A G G A A A T T  
C T G T G T T G T C T A C A G T C T A G A A T C C T T T A A 390  
370 380

ILE SER GLU ILE THR ILE ARG ASN ASP ASN  
C A T T A G T G A A A T T A C A A T T A G A A A T G A T A A  
G T A A T C A C T T T A A T G T T A A T C T T T A C T A T T 420  
400 410

GLN GLU VAL LEU PRO GLN ARG ILE THR HIS  
T C A A G A A G T G C T G C C A C A A A G A A T A A C A C A  
A G T T C T T C A C G A C G G T G T T T C T T A T T G T G T 450  
430 440

ASP VAL GLY ILE LYS PRO LEU ASN PRO ASP  
T G A T G T G G G T A T A A A A C C T T T A A A T C C A G A  
A C T A C A C C C A T A T T T T G G A A A T T T A G G T C T 480  
460 470

ASP PHE TRP ARG CYS THR SER GLY LEU PRO  
T G A T T T T T G G A G A T G C A C G T C T G G T C T T C C  
A C T A A A A A C C T C T A C G T G C A G A C C A G A A G G 510  
490 500

SER LEU MET LYS THR PRO LYS ILE ARG LEU  
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T A G A A A T T A C T T T T G A G G T T T T T A T T C C A A 540  
520 530

MET PRO GLY PRO GLY LEU LEU ALA MET PRO  
A A T G C C A G G G C C G G G A T T A T T A G C T A T G C C  
T T A C G G T C C C G G C C C T A A T A A T C G A T A C G G 570  
550 560

THR THR VAL ASP GLY CYS ILE ARG THR PRO  
A A C G A C T G T T G A T G G C T G T A T C A G A A C T C C  
T T G C T G A C A A C T A C C G A C A T A G T C T T G A G G 600  
580 590

SER LEU VAL ILE ASN ASP LEU ILE TYR ALA  
 G T C C T T A G T T A T A A A T G A T C T G A T T T A T G C  
 C A G G A A T C A A T A T T T A C T A G A C T A A A T A C G  
 610 620 630

TYR THR SER ASN LEU ILE THR ARG GLY CYS  
 T T A T A C C T C A A A T C T A A T T A C T C G A G G T T G  
 A A T A T G G A G T T T A G A T T A A T G A G C T C C A A C  
 640 650 660

GLN ASP ILE GLY LYS SER TYR GLN VAL LEU  
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 A G T C C T A T A T C C T T T T A G T A T A G T T C A G A A  
 670 680 690

GLN ILE GLY ILE ILE THR VAL ASN SER ASP  
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 T G T C T A T C C C T A T T A T T G A C A T T T G A G T C T  
 700 710 720

LEU VAL PRO ASP LEU ASN PRO ARG ILE SER  
 C T T G G T A C C T G A C T T A A A T C C C A G G A T C T C  
 G A A C C A T G G A C T G A A T T T A G G G T C C T A G A G  
 730 740 750

HIS THR PHE ASN ILE ASN ASP ASN ARG LYS  
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 A G T A T G A A A A T T G T A T T T A C T G T T A T C C T T  
 760 770 780

SER CYS SER LEU ALA LEU LEU ASN THR ASP  
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 C A G T A C A A G A G A T C G T G A G G A T T T A T G T C T  
 790 800 810

VAL TYR GLN LEU CYS SER THR PRO LYS VAL  
 T G T A T A T C A A C T G T G T T C A A C T C C C A A A G T  
 A C A T A T A G T T G A C A C A A G T T G A G G G T T T C A  
 820 830 840

ASP GLU ARG SER ASP TYR ALA SER SER GLY  
 T G A T G A A A G A T C A G A T T A T G C A T C A T C A G G  
 A C T A C T T T C T A G T C T A A T A C G T A G T A G T C C  
 850 860 870

ILE GLU ASP ILE VAL LEU ASP ILE VAL ASN  
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 G T A T C T T C T A T A A C A T G A A C T A T A A C A G T T  
 880 890 900

TYR ASP GLY SER ILE SER THR THR ARG PHE  
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 A A T A C T A C C G A G T T A G A G T T G T T G T T C T A A  
 910 920 930

LYS ASN ASN ASN ILE SER PHE ASP GLN PRO  
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 A T T C T T A T T A T T G T A T T C G A A A C T A G T T G G  
 940 950 960

TYR ALA ALA LEU TYR PRO SER VAL GLY PRO  
 T T A T G C T G C A C T A T A C C C A T C T G T T G G A C C  
 A A T A C G A C G T G A T A T G G G T A G A C A A C C T G G  
 970 980 990

GLY ILE TYR TYR LYS GLY LYS ILE ILE PHE  
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 T C C C T A T A T G A T G T T T C C G T T T T A T T A T A A  
 1000 1010 1020

LEU GLY TYR GLY GLY LEU GLU HIS PRO ILE  
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 A G A G C C C A T A C C T C C A G A A C T T G T A G G T T A  
 1030 1040 1050

ASN GLU ASN VAL ILE CYS ASN THR THR GLY  
 A A A T G A G A A T G T A A T C T G C A A C A C A A C T G G  
 T T T A C T C T T A C A T T A G A C G T T G T G T T G A C C  
 1060 1070 1080

CYS PRO GLY LYS THR GLN ARG ASP CYS ASN  
 G T G T C C C G G G A A A A C A C A G A G A G A C T G C A A  
 C A C A G G G C C C T T T T G T G T C T C T C T G A C G T T  
 1090 1100 1110

GLN ALA SER HIS SER PRO TRP PHE SER ASP  
 T C A G G C A T C T C A T A G T C C A T G G T T T T C A G A  
 A G T C C G T A G A G T A T C A G G T A C C A A A A G T C T  
 1120 1130 1140

ARG ARG MET VAL ASN SER ILE ILE VAL VAL  
 T A G G A G G A T G G T C A A C T C T A T C A T T G T T G T  
 A T C C T C C T A C C A G T T G A G A T A G T A A C A A C A  
 1150 1160 1170

ASP LYS GLY LEU ASN SER ILE PRO LYS LEU  
 T G A C A A A G G C T T A A A C T C A A T T C C A A A A T T  
 A C T G T T T C C G A A T T T G A G T T A A G G T T T T A A  
 1180 1190 1200

LYS VAL TRP THR ILE SER MET ARG GLN ASN  
 G A A G G T A T G G A C G A T A T C T A T G A G A C A G A A  
 C T T C C A T A C C T G C T A T A G A T A C T C T G T C T T  
 1210 1220 1230

TYR TRP GLY SER GLU GLY ARG LEU LEU LEU  
 T T A C T G G G G G T C A G A A G G A A G G T T A C T T C T  
 A A T G A C C C C C A G T C T T C C T T C C A A T G A A G A  
 1240 1250 1260

LEU GLY ASN LYS ILE TYR ILE TYR THR ARG  
 A C T A G G T A A C A A G A T C T A T A T A T A T A C A A G  
 T G A T C C A T T G T T C T A G A T A T A T A T A T G T T C  
 1270 1280 1290

SER THR SER TRP HIS SER LYS LEU GLN LEU  
 A T C C A C A A G T T G G C A T A G C A A G T T A C A A T T  
 T A G G T G T T C A A C C G T A T C G T T C A A T G T T A A  
 1300 1310 1320

GLY ILE ILE ASP ILE THR ASP TYR SER ASP  
 A G G A A T A A T T G A T A T T A C T G A T T A C A G T G A  
 T C C T T A T T A A C T A T A A T G A C T A A T G T C A C T  
 1330 1340 1350

ILE ARG ILE LYS TRP THR TRP HIS ASN VAL  
 T A T A A G G A T A A A A T G G A C A T G G C A T A A T G T  
 A T A T T C C T A T T T T A C C T G T A C C G T A T T A C A  
 1360 1370 1380

LEU SER ARG PRO GLY ASN ASN GLU CYS PRO  
 G C T A T C A A G A C C A G G A A A C A A T G A A T G T C C  
 C G A T A G T T C T G G T C C T T T G T T A C T T A C A G G  
 1390 1400 1410

TRP GLY HIS SER CYS PRO ASP GLY CYS ILE  
 A T G G G G A C A T T C A T G T C C A G A T G G A T G T A T  
 T A C C C C T G T A A G T A C A G G T C T A C C T A C A T A  
 1420 1430 1440

THR GLY VAL TYR THR ASP ALA TYR PRO LEU  
 A A C A G G A G T A T A T A C T G A T G C A T A T C C A C T  
 T T G T C C T C A T A T A T G A C T A C G T A T A G G T G A  
 1450 1460 1470

ASN PRO THR GLY SER ILE VAL SER SER VAL  
 C A A T C C C A C A G G G A G C A T T G T G T C A T C T G T  
 G T T A G G G T G T C C C T C G T A A C A C A G T A G A C A  
 1480 1490 1500

ILE LEU ASP SER GLN LYS SER ARG VAL ASN  
C A T A T T A G A T T C A C A A A A A T C G A G A G T G A A  
G T A T A A T C T A A G T G T T T T T A G C T C T C A C T T  
1510 1520 1530

PRO VAL ILE THR TYR SER THR ALA THR GLU  
C C C A G T C A T A A C T T A C T C A A C A G C A A C C G A  
G G G T C A G T A T T G A A T G A G T T G T C G T T G G C T  
1540 1550 1560

ARG VAL ASN GLU LEU ALA ILE ARG ASN ARG  
A A G A G T A A A C G A G C T G G C C A T C C G A A A C A G  
T T C T C A T T T G C T C G A C C G G T A G G C T T T G T C  
1570 1580 1590

THR LEU SER ALA GLY TYR THR THR THR SER  
A A C A C T C T C A G C T G G A T A T A C A A C A A C A G  
T T G T G A G A G T C G A C C T A T A T G T T G T T G T T C  
1600 1610 1620

CYS ILE THR HIS TYR ASN LYS GLY TYR CYS  
C T G C A T C A C A C A C T A T A A C A A A G G A T A T T G  
G A C G T A G T G T G T G A T A T T G T T T C C T A T A A C  
1630 1640 1650

PHE HIS ILE VAL GLU ILE ASN GLN LYS SER  
T T T T C A T A T A G T A G A A A T A A A T C A G A A A A G  
A A A A G T A T A T C A T C T T T A T T T A G T C T T T T C  
1660 1670 1680

LEU ASN THR LEU GLN PRO MET LEU PHE LYS  
C T T A A A C A C A C T T C A A C C C A T G T T G T T C A A  
G A A T T T G T G T G A A G T T G G G T A C A A C A A G T T  
1690 1700 1710

THR GLU VAL PRO LYS SER CYS SER \*\*\*  
G A C A G A G G T T C C A A A A A G C T G C A G T T A A T C  
C T G T C T C C A A G G T T T T T C G A C G T C A A T T A G  
1720 1730 1740

A T A A T T A A C C G C A A T A T G C A T T A A C C T A T C  
T A T T A A T T G G C G T T A T A C G T A A T T G G A T A G  
1750 1760 1770

T A T A A T A C A A G T A T A T G A T A A G T A A T C A G C  
A T A T T A T G T T C A T A T A C T A T T C A T T A G T C G  
1780 1790 1800

A A T C A G A C A A T A G A C A A A A G G G A A A T A T A A  
 T T A G T C T G T T A T C T G T T T T C C C T T T A T A T T  
 1810 1820 1830

A A A  
 T T T

Figure 3: Nucleotide and amino acid sequences of the PIV-3 HN gene.  
 The cDNA sequence is shown in the plus (mRNA) strand sense in the  
 5' to 3' direction. The transmembrane (TM) anchor domain is  
 underlined. Amino acids differing from the published coding  
 sequence of the PIV-3 HN gene are boxed.

FIGURE 4: RESTRICTION MAP OF THE RSV F GENE



1.0 cm = 100 bases

FIGURE 5.

MET GLU LEU **PRO** ILE LEU LYS ALA A  
 T A A C A A T G G A G T T G C C A A T C C T C A A A G C A A  
 A T T G T T A C C T C A A C G G T T A G G A G T T T C G T T  
 10 20 30

---

SN ALA ILE THR THR ILE LEU **ALA** ALA VAL T  
 A T G C A A T T A C C A C A A T C C T C G C T G C A G T C A  
 T A C G T T A A T G G T G T T A G G A G C G A C G T C A G T  
 40 50 60

---

HR PHE CYS PHE ALA **SER** SER GLN ASN ILE T  
 C A T T T T G C T T T G C T T C T A G T C A A A A C A T C A  
 G T A A A A C G A A A C G A A G A T C A G T T T T G T A G T  
 70 80 90

HR GLU GLU PHE TYR GLN SER THR CYS SER A  
 C T G A A G A A T T T T A T C A A T C A A C A T G C A G T G  
 G A C T T C T T A A A A T A G T T A G T T G T A C G T C A C  
 100 110 120

LA VAL SER LYS GLY TYR LEU SER ALA LEU A  
 C A G T T A G C A A A G G C T A T C T T A G T G C T C T A A  
 G T C A A T C G T T T C C G A T A G A A T C A C G A G A T T  
 130 140 150

RG THR GLY TRP TYR THR SER VAL ILE THR I  
 G A A C T G G T T G G T A T A C T A G T G T T A T A A C T A  
 C T T G A C C A A C C A T A T G A T C A C A A T A T T G A T  
 160 170 180

LE GLU LEU SER ASN ILE LYS GLU ASN LYS C  
 T A G A A T T A A G T A A T A T C A A G G A A A A T A A G T  
 A T C T T A A T T C A T T A T A G T T C C T T T T A T T C A  
 190 200 210

YS ASN GLY THR ASP ALA LYS VAL LYS LEU **M**  
 G T A A T G G A A C A G A T G C T A A G G T A A A A T T G A  
 C A T T A C C T T G T C T A C G A T T C C A T T T T A A C T  
 220 230 240

**ET** LYS GLN GLU LEU ASP LYS TYR LYS ASN A  
 T G A A A C A A G A A T T A G A T A A A T A T A A A A A T G  
 A C T T T G T T C T T A A T C T A T T T A T A T T T T T A C  
 250 260 270

LA VAL THR GLU LEU GLN LEU LEU MET GLN S  
 C T G T A A C A G A A T T G C A G T T G C T C A T G C A A A  
 G A C A T T G T C T T A A C G T C A A C G A G T A C G T T T  
 280 290 300



ER THR PRO ALA ALA ASN ASN ARG ALA ARG A  
G C A C A C C A G C A G C A A A C A A T C G A G C C A G A A  
C G T G T G G T C G T C G T T T G T T A G C T C G G T C T T  
310 320 330

RG GLU LEU PRO ARG PHE MET ASN TYR THR L  
G A G A A C T A C C A A G G T T T A T G A A T T A T A C A C  
C T C T T G A T G G T T C C A A A T A C T T A A T A T G T G  
340 350 360

EU ASN ASN THR LYS LYS THR ASN VAL THR L  
T C A A C A A T A C C A A A A A A C C A A T G T A A C A T  
A G T T G T T A T G G T T T T T T T G G T T A C A T T G T A  
370 380 390

EU SER LYS LYS ARG LYS ARG ARG PHE LEU G  
T A A G C A A G A A A A G G A A A A G A A G A T T T C T T G  
A T T C G T T C T T T T C C T T T T C T T C T A A A G A A C  
400 410 420

LY PHE LEU LEU GLY VAL GLY SER ALA ILE A  
G T T T T T T G T T A G G T G T T G G A T C T G C A A T C G  
C A A A A A A C A A T C C A C A A C C T A G A C G T T A G C  
430 440 450

LA SER GLY ILE ALA VAL SER LYS VAL LEU H  
C C A G T G G C A T T G C T G T A T C T A A G G T C C T G C  
G G T C A C C G T A A C G A C A T A G A T T C C A G G A C G  
460 470 480

IS LEU GLU GLY GLU VAL ASN LYS ILE LYS S  
A C T T A G A A G G A G A G T G A A C A A G A T C A A A A  
T G A A T C T T C C T T C A C T T G T T C T A G T T T T  
490 500 510

ER ALA LEU LEU SER THR ASN LYS ALA VAL V  
G T G C T C T A C T A T C C A C A A A C A A G G C C G T A G  
C A C G A G A T G A T A G G T G T T T G T T C C G G C A T C  
520 530 540

AL SER LEU SER ASN GLY VAL SER VAL LEU T  
T C A G C T T A T C A A A T G G A G T T A G T G T C T T A A  
A G T C G A A T A G T T T A C C T C A A T C A C A G A A T T  
550 560 570

HR SER LYS VAL LEU ASP LEU LYS ASN TYR I  
C C A G C A A A G T G T T A G A C C T C A A A A A C T A T A  
G G T C G T T T C A C A A T C T G G A G T T T T T G A T A T  
580 590 600

LE ASP LYS GLN LEU LEU PRO ILE VAL ASN L  
T A G A T A A A C A A T T G T T A C C T A T T G T G A A T A  
A T C T A T T T T G T T A A C A A T G G A T A A C A C T T A T  
610 620 630

YS GLN SER CYS ARG ILE SER ASN ILE GLU T  
A G C A A A G C T G C A G A A T A T C A A A T A T A G A A A  
T C G T T T C G A C G T C T T A T A G T T T A T A T C T T T  
640 650 660

HR VAL ILE GLU PHE GLN GLN LYS ASN ASN A  
C T G T G A T A G A G T T C C A A C A A A A G A A C A A C A  
G A C A C T A T C T C A A G G T T G T T T T C T T G T T G T  
670 680 690

RG LEU LEU GLU ILE THR ARG GLU PHE SER V  
G A C T A C T A G A G A T T A C C A G G G A A T T T A G T G  
C T G A T G A T C T C T A A T G G T C C C T T A A A T C A C  
700 710 720

AL ASN ALA GLY VAL THR THR PRO VAL SER T  
T T A A T G C A G G T G T A A C T A C A C C T G T A A G C A  
A A T T A C G T C C A C A T T G A T G T G G A C A T T C G T  
730 740 750

HR TYR MET LEU THR ASN SER GLU LEU LEU S  
C T T A C A T G T T A A C T A A T A G T G A A T T A T T G T  
G A A T G T A C A A T T G A T T A T C A C T T A A T A A C A  
760 770 780

ER LEU ILE ASN ASP MET PRO ILE THR ASN A  
C A T T A A T C A A T G A T A T G C C T A T A A C A A A T G  
G T A A T T A G T T A C T A T A C G G A T A T T G T T T A C  
790 800 810

SP GLN LYS LYS LEU MET SER ASN ASN VAL G  
A T C A G A A A A A G T T A A T G T C C A A C A A T G T T C  
T A G T C T T T T T C A A T T A C A G G T T G T T A C A A G  
820 830 840

LN ILE VAL ARG GLN GLN SER TYR SER ILE M  
A A A T A G T T A G A C A G C A A A G T T A C T C T A T C A  
T T T A T C A A T C T G T C G T T T C A A T G A G A T A G T  
850 860 870

ET SER ILE ILE LYS GLU GLU VAL LEU ALA T  
T G T C C A T A A T A A A A G A G G A A G T C T T A G C A T  
A C A G G T A T T A T T T T C T C C T T C A G A A T C G T A  
880 890 900

YR VAL VAL GLN LEU PRO LEU TYR GLY VAL I  
A T G T A G T A C A A T T A C C A C T A T A T G G T G T G A  
T A C A T C A T G T T A A T G G T G A T A T A C C A C A C T  
910 920 930

LE ASP THR PRO CYS TRP LYS LEU HIS THR S  
T A G A T A C A C C T T G T T G G A A A T T A C A C A C A T  
A T C T A T G T G G A A C A A C C T T T A A T G T G T G T A  
940 950 960

ER PRO LEU CYS THR THR ASN THR LYS GLU G  
C C C C T C T A T G T A C A A C C A A C A C A A A G A A G  
G G G G A G A T A C A T G T T G G T T G T T T T C T T C  
970 980 990

LY SER ASN ILE CYS LEU THR ARG THR ASP A  
G G T C A A A C A T C T G T T T A A C A A G A A C T G A C A  
C C A G T T T G T A G A C A A A T T G T T C T T G A C T G T  
1000 1010 1020

RG GLY TRP TYR CYS ASP ASN ALA GLY SER V  
G A G G A T G G T A C T G T G A C A A T G C A G G A T C A G  
C T C C T A C C A T G A C A C T G T T A C G T C C T A G T C  
1030 1040 1050

AL SER PHE PHE PRO GLN ALA GLU THR CYS L  
T A T C T T T C T T C C C A C A A G C T G A A A C A T G T A  
A T A G A A A G A A G G G T G T T C G A C T T T G T A C A T  
1060 1070 1080

YS VAL GLN SER ASN ARG VAL PHE CYS ASP T  
A A G T T C A A T C G A A T C G A G T A T T T T G T G A C A  
T T C A A G T T A G C T T A G C T C A T A A A A C A C T G T  
1090 1100 1110

HR MET ASN SER LEU THR LEU PRO SER GLU V  
C A A T G A A C A G T T T A A C A T T A C C A A G T G A A G  
G T T A C T T G T C A A A T T G T A A T G G T T C A C T T C  
1120 1130 1140

AL ASN LEU CYS ASN VAL ASP ILE PHE ASN P  
T A A A T C T C T G C A A T G T T G A C A T A T T C A A T C  
A T T T A G A G A C G T T A C A A C T G T A T A A G T T A G  
1150 1160 1170

RO LYS TYR ASP CYS LYS ILE MET THR SER L  
C C A A A T A T G A T T G T A A A A T T A T G A C T T C A A  
G G T T T A T A C T A A C A T T T T A A T A C T G A A G T T  
1180 1190 1200

YS THR ASP VAL SER SER SER VAL ILE THR S  
A A A C A G A T G T A A G C A G C T C C G T T A T C A C A T  
T T T G T C T A C A T T C G T C G A G G C A A T A G T G T A  
1210 1220 1230

ER LEU GLY ALA ILE VAL SER CYS TYR GLY L  
C T C T A G G A G C C A T T G T G T C A T G C T A T G G C A  
G A G A T C C T C G G T A A C A C A G T A C G A T A C C G T  
1240 1250 1260

YS THR LYS CYS THR ALA SER ASN LYS ASN A  
A A A C T A A A T G T A C A G C A T C C A A T A A A A A T C  
T T T G A T T T A C A T G T C G T A G G T T A T T T T T A G  
1270 1280 1290

RG GLY ILE ILE LYS THR PHE SER ASN GLY C  
G T G G A A T C A T A A A G A C A T T T T C T A A C G G G T  
C A C C T T A G T A T T T C T G T A A A A G A T T G C C C A  
1300 1310 1320

YS ASP TYR VAL SER ASN LYS GLY VAL ASP T  
G T G A T T A T G T A T C A A A T A A A G G G G T G G A C A  
C A C T A A T A C A T A G T T T A T T T C C C C A C C T G T  
1330 1340 1350

HR VAL SER VAL GLY ASN THR LEU TYR TYR V  
C T G T G T C T G T A G G T A A C A C A T T A T A T T A T G  
G A C A C A G A C A T C C A T T G T G T A A T A T A A T A C  
1360 1370 1380

AL ASN LYS GLN GLU GLY LYS SER LEU TYR V  
T A A A T A A G C A A G A A G G C A A A A G T C T C T A T G  
A T T T A T T C G T T C T T C C G T T T T C A G A G A T A C  
1390 1400 1410

AL LYS GLY GLU PRO ILE ILE ASN PHE TYR A  
T A A A A G G T G A A C C A A T A A T A A A T T T C T A T G  
A T T T T C C A C T T G G T T A T T A T T T A A A G A T A C  
1420 1430 1440

SP PRO LEU VAL PHE PRO SER ASP GLU PHE A  
A C C C A T T A G T A T T C C C C T C T G A T G A A T T T G  
T G G G T A A T C A T A A G G G G A G A C T A C T T A A A C  
1450 1460 1470

SP ALA SER ILE SER GLN VAL ASN GLU LYS I  
A T G C A T C A A T A T C T C A A G T C A A T G A G A A G A  
T A C G T A G T T A T A G A G T T C A G T T A C T C T T C T  
1480 1490 1500

LE ASN GLN SER LEU ALA PHE ILE ARG LYS S  
T T A A C C A G A G T T T A G C A T T T A T T C G T A A A T  
A A T T G G T C T C A A A T C G T A A A T A A G C A T T T A  
1510 1520 1530

ER ASP GLU LEU LEU HIS ASN VAL ASN ALA G  
C C G A T G A A T T A T T A C A T A A T G T A A A T G C T G  
G G C T A C T T A A T A A T G T A T T A C A T T T A C G A C  
1540 1550 1560

LY LYS SER THR THR ASN ILE MET ILE THR T  
G T A A A T C A A C C A C A A A T A T C A T G A T A A C T A  
C A T T T A G T T G G T G T T T A T A G T A C T A T T G A T  
1570 1580 1590

TM  
HR ILE ILE ILE VAL ILE ILE VAL ILE LEU L  
C T A T A A T T A T A G T G A T T A T A G T A A T A T T G T  
G A T A T T A A T A T C A C T A A T A T C A T T A T A A C A  
1600 1610 1620

EU SER LEU ILE ALA VAL GLY LEU LEU LEU T  
T A T C A T T A A T T G C T G T T G G A C T G C T C C T A T  
A T A G T A A T T A A C G A C A A C C T G A C G A G G A T A  
1630 1640 1650

YR CYS LYS ALA ARG SER THR PRO VAL THR L  
A C T G T A A G G C C A G A A G C A C A C C A G T C A C A C  
T G A C A T T C C G G T C T T C G T G T G G T C A G T G T G  
1660 1670 1680

EU SER LYS ASP GLN LEU SER GLY ILE ASN A  
T A A G C A A G G A T C A A C T G A G T G G T A T A A A T A  
A T T C G T T C C T A G T T G A C T C A C C A T A T T T A T  
1690 1700 1710

SN ILE ALA PHE SER ASN \* \* \*

A T A T T G C A T T T A G T A A C T G A A T A A A A A T A G  
T A T A A C G T A A A T C A T T G A C T T A T T T T T A T C  
1720 1730 1740

C A C C T A A T C A T G T T C T T A C A A T G G T T T A C T  
G T G G A T T A G T A C A A G A A T G T T A C C A A A T G A  
1750 1760 1770

A T C T G C T C A T A G A C A A C C C A T C T A T C A T T G  
T A G A C G A G T A T C T G T T G G G T A G A T A G T A A C  
1780 1790 1800

G A T T T T C T T A A A A T C T G A A C T T C A T C G A A A  
 C T A A A A G A A T T T T A G A C T T G A A G T A G C T T T  
 1810 1820 1830

C T C T T A T C T A T A A A C C A T C T C A C T T A C A C T  
 G A G A A T A G A T A T T T G G T A G A G T G A A T G T G A  
 1840 1850 1860

A T T T  
 T A A A

Figure 5: Nucleotide and amino acid sequences of the RSV F gene. The cDNA sequence is shown in the plus (mRNA) strand sense in the 5' to 3' direction. The signal peptide (SP) and the transmembrane (TM) anchor domain are underlined. The predicted F2-F1 cleavage site is indicated by the arrow ( ↓ ). Amino acids differing from the published coding sequence of the RSV F gene are boxed.

FIGURE 6: RESTRICTION MAP OF THE RSV F GENE

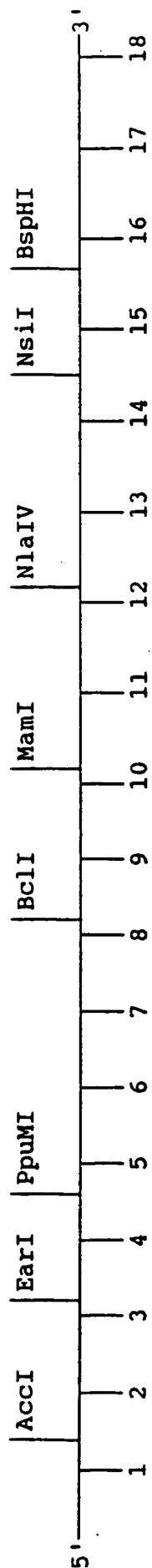


FIGURE 7: SEQUENCE OF OLIGONUCLEOTIDE CASSETTES

Fig. 7A

BsrI BamHI  
 5' ---ATCAATCAAAGGTCCTGTGATAATAG---- 3'  
 CGTAGTTAGTTTCCAGACACTATTATCCTAG

Fig. 7B

BspHI BamHI  
 5' CATGACTTGATAATGAG---- 3'  
 ----TGAACATATTACTCCTAG

Fig. 7C

EcoRI PpuMI  
 5' AATTCATGGAGTTGCTTAATCCTCAAAGCAAATGCAATACCAATCCTCAGTCAGTCACATTTTGTGCTTCTGGTTCTAAG---- 3'  
 ----GTACCTCAACGATTAGGAGTTTCGTTACGTTAATGGTGTAGGAGTGACGTCAGTGTAAACAAAGCAAGACCAGATTCCAG

Fig. 7D

BsrI BamHI  
 5' ACTGGCATCAATCTAGCAGTACATGAG---- 3'  
 ----CGTAGTTAGATCGTGATGTACTCCTAG

Fig. 7E

EcoRI BstBI  
 5' AATTCATGCCAACTTTAATACTGCTAATTATTACAAATGATTATGGCATCTTCTGCGCAATAGATATCACAAACTACAGCATGTAGGTGTATTGGTCAACAGTCCC  
 ----GTACGGTTGAATTATGACGATTAAATGTTGTACTAATACCGTAGAAGGACGGTTTATCTATAGTCTTTTGTATGTCCTACATCCACATAACCCAGTTGTCCAGGG  
 AAAGGATGAAGATATCAAAAACTT----  
 TTTCCCTACTTCTATAGTGTTTTGAAGCTT



FIGURE 8:

SEQUENCE OF OLIGONUCLEOTIDE CASSETTE USED TO RESTORE NATIVE

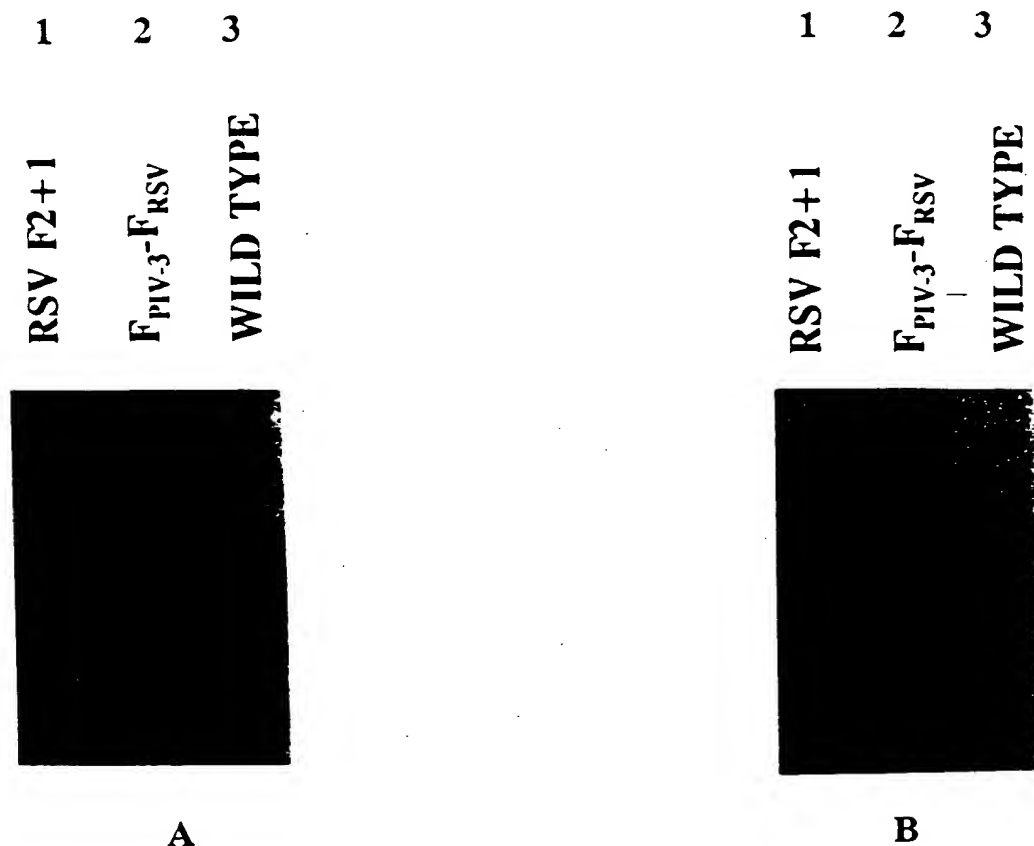
POLYHEDRIN PROMOTER IN THE PAC 610 VECTOR

EcoRV

EcoRI

ATCATGGAGATAATTAAATGATAACCATCTCGCAATAAATAAGTATTTTACTGTTTTTCGTAACAGTTTTTGTAATAAAAAAACCTATAAATAG-----  
TAGTACCTCTATTAAATTTTACTATTGGTAGAGCGTTTTATTATTTCATAAAATGACAAAAAGCATTTGTCAAAACATTAATTTTTTTTGGATATTTTATCTTAA

# **FIGURE 9: IMMUNOBLOTS OF CELL LYSATES FROM Sf9 CELLS INFECTED WITH RECOMBINANT BACULOVIRUSES**



**Figure 9:** Immunoblots of cell lysates from Sf9 cells infected with recombinant baculoviruses containing the truncated RSV F gene (Lane 1), the chimeric F<sub>PIV-3</sub>-F<sub>RSV</sub> gene (Lane 2) or infected with wild type virus (Lane 3) reacted with anti-F RSV Mab (panel A) and anti-F1 PIV-3 antiserum (panel B).

**FIGURE 10: SEQUENCE OF OLIGONUCLEOTIDE CASSETTE**

**BspHI**

**BspHI**

**CATGACTAATTCCATCAAAAGTGAAAAGGCT----**  
**-----TGATTAAGGTAGTTTTCACTTTTCCGAGTAC**